EVALUATION OF A SOLID-PHASE IMMUNOBEAD ASSAY FOR DETECTION OF CIGUATERA-RELATED BIOTOXINS IN CARIBBEAN FINFISH

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The Ciguatect™ solid-phase immunobead assay for detection of ciguatera-related polyether biotoxins in finfish was evaluated for consistency with the mouse bioassay. Fifty finfish from ciguatera-endemic waters of St. Thomas, U.S. Virgin Islands, and one fish remnant from a confirmed case of ciguatera poisoning were mouse bioassayed. The 51 specimens were then assayed using the Ciguatect™ assay with 3 different methods of tissue sampling: single exposure, triple exposure and single exposure to solvent extract from flesh (REM™: rapid extract method). Qualitative statistical analyses ascertained false positive and false negative rates. Positive matches for the single, triple and REM™ methods of tissue sampling were 58, 85 and 94%, respectively, and negative matches were 17, 22 and 12%, respectively. Corresponding false negative rates were 82, 55 and 50%, and false positive rates were 44, 33 and 33%. Predictive indices for Ciguatect ™ performance under ciguatoxin contamination rates ranging from 5 to 75% project that high false negative and false positive values might be expected in market situations.

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The major impediment to management strategies for the ciguatera public health hazard is the difficulty in detecting highly potent ciguatoxins (and maitotoxins) in seafood matrices. Ciguatoxin and maitotoxin structures have been elucidated from Pacific sources (Murata et al., 1989, 1990,1992,1993; Lewis et al.,1991; Yokoyama et al.,1988; Holmes et al., 1990), but Caribbean forms have not been resolved and analytical methods to detect and quantify them are not yet available. Several potential assessory toxins have also been characterized (Murakami et al.,1982; Torigoe et al.,1988; Nagai et al.,1992; Fukui et al., 1987). Difficulties with developing ciguatera detection methods are due to lack of analytical standards. An immunochemical assay for detection of ciguatera-related polyethers was developed (Hokama,1985,1990; Hokama et al.,1990, 1992) and modified into a 'kit' format, 'Ciguatect™' (Park et al.,1992). We compare this assay with the mouse bioassay, the most widely recognized method for identification of ciguatoxic finfish.

EXPERIMENTAL

APPARATUS

Eberbach 8017 explosion-proof blender (Eberbach Corp., Ann Arbor, MI); Brinkmann/Buchi rotary evaporator Model RE-111 (Brinkmann Instruments, Inc., Westbury, NY); Mettler Model PM2000 top-loading balance (Mettler Instrument Corp., Hightstown, NJ); Sartorius Model R180D analytical balance (Sartorius Corp., Bohemia, NY); Savant Model SS-2 centrifugal evaporator (Savant Instruments, Inc., Farmingdale, NY).

REAGENTS AND MATERIALS

Solid-phase immunobead assay (SPIA) materials were purchased from Hawaii Chemtect International, Inc. (San Diego, CA) on 24 May 1992 (immunobead lot number 051492) and again on 6 October 1992 (immunobead lot number 100592). All solvents were of reagent grade or better (J.T. Baker Chemical Co., Phillipsburg, NJ). Silica gel solid-phase extraction columns were obtained from Varian Associates, Inc. (Sunnyvale, CA). Swiss white mice (Crl:CFW (SW)BR) were obtained from Charles River Laboratories (Wilmington, MA).

SPECIMEN COLLECTION

Potentially ciguatoxic finfish were collected 4–13 June,1992 from ciguatera-endemic waters S of St. Thomas, U.S. Virgin Islands (McMillan et al.,1983). Fish were solicited from local sports fishermen. Fish were collected by spearfishing. Participants in an annual fishing tournament were

notified of the need for specimens, and donations of fish were accepted at the weigh-in station. Larger fish of suspect species from areas noted for ciguatera were procured as they have the highest probability of being ciguatoxic. This approach was taken knowing that a significant percentage of finfish are non-ciguatoxic even from high incidence areas. Specimens were identified, weighed, tagged, frozen and air-freighted to the FDA Gulf Coast Seafood Laboratory (GCSL), Dauphin Island, AL. All specimens arrived frozen with no evidence of thawing or decomposition. Fish specimens were stored at -20°C.

TEST SAMPLE PREPARATION

Fifty Caribbean fish were selected, including *Sphyraena barracuda* (70%), *Caranx latus* (20 %), *C. hippos* (2%), *Seriola dumerili* (4%), *Seriola rivoliana* (2%) and *Scomberomorus cavalla* (2%). From each specimen two muscle tissue samples were taken from the anterolateral part of the body immediately behind the head: 10g for use in the SPIA procedure and 450g for extraction and mouse bioassay. Samples also were taken from a fillet portion of barracuda (specimen VI-108: courtesy of Dr. Norbert Mantor, St. Thomas Hospital) that was responsible for ciguatera poisoning in St. Thomas and from a *Cynoscion arenarius* (white trout: specimen 92-07-1; not included in the tables) obtained from the retail seafood market of Dauphin Island, AL, as a negative control. A portion of the latter specimen was consumed without toxic effect. The 10g samples were frozen at -20°C. The 450g samples were weighed to the nearest 0.1g, and extracted and partitioned (McMillan et al., 1983; Yasumoto et al.,1984). The dried products from the extraction and partitioning procedures were dissolved in chloroform and applied to 60mL (10g) silica gel solid-phase extraction (SPE) columns that had been preconditioned by washing with 60mL of the same solvent. The SPE columns were washed with an additional 120mL chloroform and then eluted with 120mL 10% methanol in chloroform. The eluting solvent mixtures were removed by rotary evaporation, and residues transferred in methanol (c.2–3mL) to tared 13x100mm Teflon-capped test tubes. Solvent was removed again by vacuum centrifugation and residue weights were recorded to the nearest 0.1mg for each final product. Final SPE products were dissolved in methanol (c.1–2mL) and stored at -20°C.

MOUSE BIOASSAYS

Methanol was removed from the muscle tissue SPE products by vacuum centrifugation. The residues were redissolved in known total volumes of saline (0.15M NaCl) containing 1% Tween-60. Appropriate aliquots of the saline solutions were taken such that the dosing of mice was equal to muscle tissue fresh weight equivalents (MTE) of 45, 90 or 180g per mouse. Residue weights administered to mice did not exceed 20mg, and ranged as low as 318μg when adjusted to the chosen MTE. All bioassay solutions were adjusted to a total injection volume of 0.5mL prior to bioassay. The 0.5mL solutions were administered by intraperitoneal injection into Swiss white mice (Crl:CFW(SW)BR) weighing 18-21g. Control mice were administered saline-Tween solution only. The mouse bioassays were performed in duplicate for controls and for each MTE of the final SPE products. Mice were observed post-injection for a period of 48hr. Signs of toxicity were noted and when death occurred, times from injection were recorded. Mouse bioassays were conducted according to the principles provided in the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animals Resources, National Research Council, NIH Pub. No. 85-24.

CIGUATECT ™ SPIA

A 9x10mm white membrane, affixed to a 90x9mm plastic strip is exposed to muscle tissue taken from behind the head of the fish. The membrane is permitted to air-dry and is then immersed in methanol for no more than 1sec. After the membrane is completely dry, it is immersed in an anti-ciguatoxin-antibody-latex bead suspension (gently mixed prior to use) for 5 or 10min. The latter solution is blue. The membrane is then dipped in and out of a phosphate-buffered saline solution 3 times, placed flat on an absorbent towel and gently blotted to remove excess saline solution. Adsorption of ciguatera biotoxins to the membrane and subsequent conjugation with the anti-ciguatoxin antibody bound to the surface of the blue latex beads should produce a blue color (cast) in the membrane affixed to the plastic strip if the fish contains ciguatera biotoxins. Kit positive-control membrane strips are processed from the point of exposure to antibody-latex bead solution. Kit negative-control membrane strips are processed from the point of exposure to methanol. All Ciguatect ™ SPIA materials are stored at 4°C.

Instructions for the first set of materials (received 24 May 1992) directed the user to expose the membranes to fish tissue only one time

TABLE 1. Mouse bioassay dosing, fish muscle tissue equivalents (MTE) and survival times. * indicates dose of 180g MTE administered to mice.

Specimen	45g MTE dose (mg/kg)	45g MTE survival time (hr)	90g MTE dose (mg/kg)	90g MTE survival time (hr)
V1-13	46.0 42.6	>48 >48	83.9 77.9	>48 >48
VI-17			1018.9 856.9	0.20* 0.36*
VI-26			369.0 337.8	25.00 >48
V1-27			258.7 236.8	3.60 1.80
VI-32			301.0 275.4	>48 >48
V1-33			183.8 219.3	0.33 0.32
V1-34			255.4 230.6	1.9 13.00
VI-35			265.2 250.5	1.00 1.56
VI-37			295.7 365.3	0.55 0.63
VI-38	122.5 119.3	0.33 0.63		
V1-48	73.3 65.3	>48 >48	121.3 130.7	>48 2.21
VI-49			80.3 68.6	0.15 0.15
V1-52	17.4 15.8	0.66 0.45	34.4 35.6	>1.00 >1.00
V1-53			82.1 79.8	2.50 6.40
V1-54	7.9 8.7	>48 >48	15.2 14.9	>48 >48
V1-55	135.8 124.4	>48 >48	218.0 229.2	>48 >48
V1-57	56.8 53.0	0.50 0.45		
V1-58	63.4 75.3	>48 >48	126.0 149.4	0.61 0.30
V1-59			123.6 121.8	>48 >48
V1-60	73.2 73.6	>48 >48	133.3 141.8	>48 >48
V1-61	131.5 133.0	3.0 >48		
V1-62	74.9 76.1	6.10 >48		
V1-63	87.4 90.5	>48 >48	157.7 154.5	>48 >48
V1-64	175.6 170.4	15.50 >48		
V1-65	81.7 83.4	>48 >48	162.2 146.9	16.00 >48
V1-67	51.5 51.8	1.51 >48	107.3 113.8	0.92 0.91
V1-70			195.3 210.8	>48 >48
V1-71	33.3 34.1	>48 >48	62.9 70.2	2.50 14.25
V1-72	35.9 31.6	>48 >48	62.1 63.7	>48 >48
V1-73	41.1 40.2	>48 >48	73.1 72.1	1.10 4.66
V1-75	110.6 120.3	>48 >48	202.3 218.4	>48 >48
V1-76	44.1 39.7	>48 >48	81.8 79.0	>48 1.68
V1-77	52.7 45.4	0.66 0.63		
V1-79	84.2 83.3	2.13 >48		
V1-81	69.7 61.4	>48 >48	131.9 128.5	>48 >48
V1-85	44.1 41.3	2.92 5.50	93.8 92.8	1.21 1.61
V1-86	54.9 56.4	28.00 4.50		
VI-88			236.3 238.7	>48 >48
V1-90	44.1 40.1	15.50 >48		
V1-91	67.0 66.7	1.53 >48		
V1-92	27.4 26.2	>48 >48	50.8 45.5	>48 >48
V1-93	70.7 71.5	>48 >48	127.2 127.2	>48 >48
V1-97	74.5 74.1	>48 >48	128.3 130.1	>48 >48
V1-98	78.0 71.1	>48 >48	123.0 129.1	>48 >48
V1-99	61.3 61.3	0.86 0.88	138.8 123.1	0.10 0.23
V1-100			54.1 54.4	>48 >48
V1-102			46.2 44.9	>48 >48
V1-105			288.1 278.6	0.13* 0.55*
V1-106	64.0 62.3	>48 >48	120.2 124.6	0.15 0.15
V1-107	54.3 48.7	>48 >48	90.9 90.0	1.10 0.73
V1-108	55.6 55.6	>48 >48	146.0 153.7 400.0 412.6	0.83 1.83 0.35* 0.22*

Specimen	single exposure		triple exposure		REM™ duplicates*	Mouse bioassay
	Mean	SD	Mean	SD		
VI-13	**1.5**	0.5	0.2	0.4	**3,3**	N
VI-17	1.8	0.4	1.8	0.4	**0,1**	T
VI-26	2.3	0.5	2.3	0.5	5,5	T
VI-27	1.8	0.4	4.0	0.0	5,6†	T
VI-32	**1.8**	0.4	**3.8**	1.2	**3,3**	N
VI-33	3.2	0.7	3.2	0.4	4,4	T
VI-34	2.5	0.5	3.8	0.4	3,3	T
VI-35	2.0	0.0	3.0	0.6	5,5	T
VI-37	3.7	0.5	2.0	0.0	4,5	T
VI-38	**0.5**	0.5	3.7	0.5	5,5	T
VI-48	**0.7**	0.5	1.8	0.4	3,3	T
VI-49	1.5	0.5	**0.7**	0.5	2,2	T
VI-52	**0.5**	0.5	3.7	0.9	2,2	T
VI-53	1.8	0.4	2.7	1.1	3,3	T
VI-54	**1.8**	0.4	**2.5**	0.5	**3,3**	N
VI-55	**2.3**	0.5	**1.2**	0.4		N
VI-57	**1.0**	0.0	2.7	1.2	5,5	T
VI-58	**0.8**	0.4	3.3	1.1	5,6†	T
VI-59	**2.2**	0.4	**2.7**	1.1	**5,6†**	N
VI-60	**2.0**	0.0	**3.8**	1.3	5,5	N
VI-61	1.5	0.5	1.8	0.4	2,2	T
VI-62	**1.0**	0.0	2.3	0.5	**1,1**	T
VI-63	**2.5**	0.5	**2.7**	0.7	1,1	N
VI-64	1.8	0.4	3.0	0.8	4,4	T
VI-65	**0.2**	0.4	**0.3**	0.5	4,5	T
VI-67	1.7	0.7	3.0	0.6	4,5	T
VI-70	**3.0**	0.6	**2.3**	0.5	**3,4**	N
VI-71	**0.7**	0.5	1.3	0.5	5,5	T
VI-72	**1.8**	0.4	**1.3**	0.7	**4,5**	N
VI-73	**0.8**	0.4	**0.0**	0.0	5,5	T
VI-75	**1.3**	0.5	**1.2**	0.4	**4,4**	N
VI-76	**1.0**	0.0	**1.0**	0.0	2,3	T
VI-77	1.7	0.7	1.2	0.4	3,3	T
VI-79	2.0	0.0	1.3	0.5	5,5	T
VI-81	0.8	0.4	1.0	0.0	**4,5**	N
VI-85	1.7	0.7	2.0	0.6	5,5	T
VI-86	3.5	1.0	2.5	0.8	5,5	T
VI-88	**3.3**	0.5	**2.0**	0.0	**4,4**	N
VI-90	**0.3**	0.5	1.8	0.4	3,3	T
VI-91	**0.3**	0.5	1.5	1.6	4,4	T
VI-92	0.7	0.5	0.7	0.5	**4,5**	N
VI-93	**2.2**	0.4	**1.5**	0.5	**3,3**	N
VI-97	**2.0**	0.0	0.0	0.0	**5,5**	N
VI-98	**1.3**	0.7	**1.3**	0.7	**2,2**	N
VI-99	1.8	0.4	**0.8**	0.4	2,3	T
VI-100	**1.2**	0.7	**2.5**	1.0	0,1	N
VI-102	0.5	0.5	**2.2**	0.4	**3,3**	N
VI-105	**0.8**	0.4	2.7	1.7	4,4	T
VI-106	1.2	0.7	1.8	0.4	2,2	T
VI-107	2.0	0.0	1.5	0.5	2,2	T
VI-108	**0.5**	0.5	2.2	0.4	5,5	T

TABLE 2. Ciguatect™ SPIA mean scores (n=5) and standard deviations (SD) for three variations of procedure. Boldface values indicate false positive or false negative scores relative to mouse bioassay results (T = ciguatoxic; N = survival beyond 48hr). * indicates data set provided by Dr D.L. Park, Univ. of Arizona. †indicates apparent inconsistency with regard to immunoassay scoring scale.

(single exposure) and to allow the membrane to dry before proceeding. Weak color development prompted the manufacturer to modify the procedure for the second set of materials (received 6 October 1992). The latter procedure instructed the user to expose the membranes by contact with fish tissue three times (triple exposure) with drying periods following each exposure. Consequently, Ciguatect™ immunoassays were performed twice on each 10g test sample. The instructions for immunoassay setup, fish assay and use of positive and negative kit controls were followed precisely. Positive and negative control test strips (provided with SPIA materials) were developed prior to fish tissue assays. The SPIA color developments for controls and fish tissues were read and interpreted independently by 6 analysts. Participants were asked to assign a value of 0 (no color) to 5 (strong color) to color development of each test strip by comparison with a standard series of positive and negative control test strips supplied by the kit manufacturer.

The 10g test samples that were used for Ciguatect™ SPIA evaluation at the FDA GCSL were frozen and delivered 'blind' (i.e., each test portion was identified only by code number) to the laboratory of the kit manufacturer for a repetition of the SPIA procedures.

STATISTICAL ANALYSIS

The Ciguatect™ SPIA method was assessed using procedures for evaluating screening tests (Fleiss,1981). Method performance is described through four interrelated rates: sensitivity, specificity, false positive, and false negative. The sensitivity rate (P_{+}) is the proportion of correctly

TABLE 3. Ciguatect™ SPIA performance rates relative to mouse bioassay results. * indicates data set provided by Dr D.L. Park, University of Arizona.

Performance criterion	Performance rate		
	Single-exposure immunoassay	Triple-exposure immunoassay	REM™ immuno-assay*
SENSITIVITY	19/33 (58%)	28/33 (85%)	31/33 (94%)
SPECIFICITY	3/18 (17%)	4/18 (22%)	2/17 (12%)
FALSE NEGATIVE	14/17 (82%)	5/9 (55%)	2/4 (50%)
FALSE POSITIVE	15/34 (44%)	14/42 (33%)	15/46 (33%)

classified 'known' positive test samples. Specificity rate (P_-) is the proportion of correctly classified 'known' negative test samples. False positive rate (PF_+) is the proportion of positive classified test samples that are misclassified 'known' negatives. False negative rate (PF_-) is the proportion of negative classified test samples that are misclassified 'known' positives. A 'known' positive or 'known' negative test sample is a test sample that has been classified as positive or negative by the reference method (i.e., mouse bioassay). False positive and false negative rates, as defined by Fleiss, are predictive rates. These were used to assess the Ciguatect™ SPIA method when used on populations of fish with different proportions of ciguatoxic specimens.

RESULTS AND DISCUSSION

Mouse Bioassay

Characteristic signs of ciguatoxicity (Hoffman et al.,1983; Kimura et al.,1982) and death within 48hr were chosen as the determinants for the presence of ciguatera-related biotoxins. The first three fish SPE products bioassayed were administered at doses of 180g MTE. Survival times were short for the three products (≤0.36hr). All subsequent bioassays were conducted at an MTE of either 90 or 45g in order to record the presence or absence of characteristic signs of ciguatoxicity. The mouse bioassays resulted in 33/51 (65%) mortalities (Table 1). A total of 79 pairs of mice were injected (duplicate bioassay for each MTE). In 10 of the 79 pairs 1 mouse expired within 48hr, and the other survived beyond 48hr. Signs of ciguatoxicity in mice included inactivity; piloerection; vasodilation in ears; cyanosis of tail, feet and muzzle; lacrimation; salivation; diarrhea; dyspnea; unsteady gait; tremor/convulsive jumping; straub tail; convulsions; and death. Not all mice displayed each of these signs of toxicity. Some characteristic signs of toxicity were not observed in mice that expired within 1hr (15/33: 45%). In 50% of short survivals the dosage was reduced to 45g MTE (lowest dose level administered) and longer survival times or survivals through 48hr were noted with characteristic signs of toxicity. The mouse bioassay of specimen VI-108 resulted in survival beyond 48hr at 45g MTE, survival times of 0.83 and 1.83 at 90g MTE, and survival times of 0.35 and 0.22 at 180g MTE. Specimen 92-07-1 resulted in survival beyond 48hr at 180g MTE without any sign of toxicity. Fish specimens from which corresponding SPE products produced mouse death with characteristic signs of toxicity at 90g MTE were considered ciguatoxic.

Ciguatect™ SPIA

SPIA ratings of the fish specimens were scaled from 0 to 5 by comparison of color development with a standard series of pre-developed membranes supplied by the manufacturer. The manufacturer specified that ratings of 0 or 1 were to be considered non-ciguatoxic and ratings above 1 through 5 ciguatoxic at progressively greater levels. The means and standard deviations of ratings for SPIA responses were recorded (Table 2). However, for comparison with mouse bioassays the specimens were scored as either positive (ciguatoxic) or negative (non-ciguatoxic) by SPIA. Results from single exposure of test-strip membranes to muscle tissues indicated that 34/51 (67%) of fish specimens were ciguatoxic. Based upon the precedent (McMillan et al.,1983; Yasumoto et al.,1984; Hoffman et al., 1983; Kimura et al.,1982; Vernoux et al., 1985) that the reference mouse bioassay accurately reflects ciguatoxicity, results of the 3 sample treatment methods with the immunoassay technique are shown in Table 3. Single-exposure SPIA scored the human illness case sample (specimen VI-108) negative (mean 0.5, SD=0.5) and the non-toxic white trout specimen positive (mean 2.7, SD=0.7). The SPIA results following triple exposure of test-strip membranes to muscle tissues indicated that 42/51 (82%) of the fish specimens were ciguatoxic. Triple-exposure SPIA scored VI-108 (mean 2.2, SD=0.4) and the white trout specimen (mean 4.0, SD=0.5) both positive. Mean scores for the kit internal controls for single exposure of test strips included 2/2 positive matches and 2/2 negative matches. Scores for kit internal controls for triple exposure

TABLE 4.Predictive indices of Ciguatect™ performance, given ciguatoxins contamination rates from 5 to 75%. PF_+ = false positive; PF_- = false negative.

Contamination (%)	Predictive index					
	Single exposure		Triple exposure		REM™	
	PF_+	PF_-	PF_+	PF_-	PF_+	PF_-
5	0.9649	0.1181	0.9457	0.0346	0.9486	0.0264
15	0.8913	0.3100	0.8386	0.1074	0.8462	0.8333
25	0.8128	0.4590	0.7333	0.1852	0.7444	0.1466
35	0.7288	0.5782	0.6300	0.2685	0.6432	0.2172
45	0.6389	0.6756	0.5284	0.3581	0.5426	0.2965
55	0.5422	0.7566	0.4286	0.4545	0.4426	0.3864
65	0.4380	0.8254	0.3305	0.5587	0.3432	0.4889
75	0.3254	0.8842	0.2340	0.6716	0.2444	0.6071

of test strips included 2/2 positive matches, 1/2 negative matches and 1 false positive (mean of 1.2 on 5-point scale). False negatives were not observed among the kit control test strips.

The Ciguatect ™ SPIA results obtained by the manufacturer's laboratory for the fish test samples evaluated by FDA GCSL do not correspond to results obtained by GCSL. The manufacturer laboratory first performed extraction and partitioning procedures (REM™ :rapid extraction method) on each test sample. The SPIA was then performed on the REM™ products (Tables 2, 3). SPIA results obtained by following the REM™ modification of the procedure indicated that 46/50 (92%) of the fish specimens were ciguatoxic. VI-108 scored positive (duplicate scores of 5 and 5).

The sensitivity and specificity test performance rates for each variation of the SPIA procedure (Table 3) were used to evaluate how the Ciguatect™ SPIA would be expected to perform when used on fish populations with different proportions of ciguatoxic specimens. Table 4 presents a summary of the predictive values for each SPIA method by population ciguatoxicity rate. Assuming that a true ciguatoxins contamination rate of 55% is encountered in a hypothetical lot of tropical fish, the predictive indices generated from the present study indicate that single-exposure Ciguatect™ SPIA would produce a false negative rate of 76% and a false positive rate of 54%. Triple-exposure Ciguatect™ SPIA would produce a false negative rate of 45% and a false positive rate of 43%, and the REM Ciguatect™ SPIA would produce a false negative rate of 39% and a false positive rate of 44%.

The Ciguatect™ SPIA was examined for interference from potential fish decomposition products and for possible non-specific toxicity unrelated to ciguatera in the mouse bioassay. A repeat sampling of 13 muscle tissues (25%) from the original 51 fish provided a cross-section of tissue that were SPIA positive and negative, mouse bioassay positive and negative, and SPIA false positive and false negative. The tissues were analyzed for putrescine and cadaverine by the method of Staruszkiewicz & Bond (1981) and for histamine by the fluorometric method (AOAC, 1990). Determination of putrescine was not possible because of matrix interference. Cadaverine levels were below the lowest calibration standard of 0.5μg/g in 10 of the 13 tissues. Three of the tissues contained 3.7, 7.2 and 8.4μg/g cadaverine (Table 5). Cadaverine levels above 6μg/g in tunafish indicate decomposition. Tolerance levels for other species of fish (including those used in the present study) are not established. Histamine levels did not exceed 0.1mg% in 11 of the 13 tissues, and did not exceed 0.2mg% in the two remaining tissues. The defect action level for histamine in the United States is 20mg%, and 50mg% poses a human health hazard. There was no correlation between cadaverine or histamine and SPIA or mouse bioassay findings.

Thirteen tissue extract products (corresponding to the specimens analyzed for decomposition products) were evaluated for sodium channel activity by using a tetrazolium-based neuroblastoma cell bioassay for neurotoxins active on sodium channels (Manger et al.,1993). Sodium channel potentiating activity is indicative of the ciguatoxins. The bioassay indicated that sodium channel activity was present in all 13 tissue products and provided a general ranking of sodium channel activity for those tested (Table 5). The activity ranking correlated well with mouse bioassay survival times. Specimen extract products shown to be highly toxic by the mouse bioassay also produced significant reductions in cell viability through sodium channel potentiating effects. Inhibitory doses which reduced cell viability by 90% after 22 hr exposure (ID_{90}) ranged from 2 to 6mg MTE per 200microliter cell culture (96μL well format). Specimen extract products classified non-toxic by the mouse bioassay were found to possess sodium channel effects at ID_{90} dosing that ranged from 7 to 40mg MTE. The latter finding, if considered in conjunction with mouse bioassay results from the ciguatera case specimen VI-108 (i.e., mouse survival at 45g MTE and expiration at 90g MTE), suggests that utilization of 90g MTE in the mouse bioassay may approximate a correlate for human toxicity.

TABLE 5. Sodium channel activity of specimen solid-phase cxtraction products (ID_{90} =inhibitory dose which reduces cell viability by 90% at 22hr exposure). Values expressed in milligram muscle tissue equivalents per well; mouse bioassay survival times; Ciguatect™ SPIA scores and decomposition indicators. * indicates data provided by Dr D.L. Park, University of Arizona. † indicates apparent inconsistency with regard to scoring scales. # indicates dose of 45g MTE administered to mice.

Specimen	Sodium channel ID_{90} (mg MTE/well)	90g MTE mouse bioassay TD (hr)	Ciguatect™ score			Decomposition products	
			Single-exposure (SD)	Triple-exposure (SD)	REM*	Cadaverine µg/g	Histamine mg%
VI-13	>40	>48 >48	1.5(0.5)	0.2(0.4)	3,3	<0.5	0.1
VI-59	32	>48 >48	2.2(0.4)	2.7(1.1)	5,6†	<0.5	0.1
VI-54	22	>48 >48	0.3(0.5)	2.7(0.7)	1,1	<0.5	0.1
VI-32	20	>48 >48	1.8(0.4)	3.8(1.2)	3,3	<0.5	0.1
VI-81	9	>48 >48	0.8(0.4)	1.0(0.0)	4,5	7.2	0.2
VI-54	7	>48 >48	0.8(0.4)	2.5(0.5)	3,3	<0.5	0.1
VI-73	6	1.10 4.66	0.8(0.4)	0.0(0.0)	5,5	<0.5	0.1
VI-65	9	16.00 >48	0.[illegible](0.[illegible])	0.3(0.5)	4,5	<0.5	0.1
VI-53	2	2.50 6.40	1.8(0.4)	2.7(1.1)	3,3	8.4	0.2
VI-85	2	1.21 1.61	1.7(0.7)	2.0(0.6)	5,5	<0.5	0.1
VI-77	2	0.66 0.63	1.7(0.7)	1.2(0.4)	3,3	<0.5	0.1
VI-38	2	0.33# 0.63#	0.5(0.5)	3.7(0.5)	5,5	<0.5	0.1
VI-57	2	0.50# 0.45#	1.0(0.0)	2.7(1.2)	5,5	3.7	0.1

CONCLUSION

Ciguatect™ SPIA performance with ciguatoxic Caribbean finfish may be characterized by low specificity rates and high false positive and false negative values. Extrapolating these performance characteristics to a market situation implies that a proportion of wholesome fish might falsely be identified as ciguatoxic and an equally significant proportion of ciguatoxic fish might reach the marketplace undetected. Conclusions of the present study cannot be extended to Ciguatect™ SPIA performance with Pacific Ocean finfish (where the immunoassay originated) without separate evaluation of the method using fish from the Pacific region.

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